MICROBIAL QUALITY OF FRESH-CUT ICEBERG LETTUCE WASHED IN WARM OR COLD WATER AND IRRADIATED IN A MODIFIED ATMOSPHERE PACKAGE*

KATHLEEN T. RAJKOWSKI[1] and XUETONG FAN

United States Department of Agriculture, Agricultural Research Service
Eastern Regional Research Center
600 E. Mermaid Lane, Wyndmoor, PA 19038

Accepted for Publication April 30, 2007

ABSTRACT

The microbial keeping quality of fresh-cut iceberg lettuce was determined after being washed in either cold water (5C) for 3 min or warm water (47C) for 2 min followed by a cold water rinse (5C) and packaged in a modified atmosphere film bag. The lettuce samples were treated with gamma radiation to 0, 1 or 2 kGy while maintaining a refrigerated temperature (4C). The samples were analyzed for total aerobic, total coliform and Enterobacteriaceae counts after refrigerated storage up to 12 days. No difference in aerobic counts was observed between the hot- and cold-washed samples immediately after washing. The coliform and Enterobacteriaceae counts were reduced by 2 log after the warm water wash and no difference for the cold water-washed sample. The irradiation treatment at 1 kGy reduced the aerobic, coliform and Enterobacteriaceae counts by 2 log for the warm-washed samples. At the 2-kGy treatment level, the aerobic and coliform counts were reduced by 3 log for the cold-washed lettuce, whereas the Enterobacteriaceae counts were reduced by only 2 log. The observed log reductions in bacterial counts after irradiation were maintained for 12 days when stored at 4C. The combination of a cold water wash and irradiation to 2 kGy had the best microbial keeping quality.

PRACTICAL APPLICATIONS

Fresh-cut lettuce, when washed in either cold or warm water, shows neither an appreciable removal of the microbial load nor a significant increase

* Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture.

[1] Corresponding author. TEL: 215-233-6440; FAX: 215-233-6406; EMAIL: krajkowski@errc.ars.usda.gov

Journal of Food Safety **28** (2008) 248–260.

in the keeping quality when compared with unwashed fresh-cut controls. Placing the washed lettuce into modified atmosphere packaging (MAP) did not lessen the overall bacteriologic load, and after 12 days of storage at 4C, the microbial counts increased. However, gamma irradiation of the washed, MAP-stored lettuce to a dose of 2 kGy significantly reduces the overall microbe count, thereby increasing both the shelf life and the safety of the produce. A 2-kGy dose of gamma irradiation provides a pathogen-free, long shelf life, fresh-cut lettuce that is bacteriologically safer and sensorially indistinguishable from bagged, nonirradiated, fresh-cut lettuce.

INTRODUCTION

The per capita use of all lettuce products in the U.S.A. has steadily increased (United States Department of Agriculture, Economic Research Service 2005). At one time, fresh fruits and vegetables were not considered food products that could support or be contaminated with human pathogens. However, this attitude has now changed. In a review of pathogenic microorganisms associated with raw vegetables, Beuchat (1996) listed those human pathogens that could survive and grow on lettuce and salad greens. There are confirmed foodborne outbreaks associated with the consumption of fresh lettuce products contaminated with *Escherichia coli* O157:H7 (Ackers *et al.* 1998; Hilborn *et al.* 1999). With the awareness of confirmed outbreaks associated with lettuce products and increased consumption, loose-leaf lettuce was one of the produce commodities included in the U.S. Department of Health and Human Services, Food and Drug Administration (FDA) (2003a,b) domestic and imported fresh produce survey of 2000. The FDA reported that 2 out of 116 imported lettuce samples tested were pathogen positive (U.S. Department of Health and Human Services, Food and Drug Administration 2003b) and one out of 142 domestic lettuces (U.S. Department of Health and Human Services, Food and Drug Administration 2003a) was positive for *Salmonella*. The contamination with human pathogens can occur during the growth of the produce using bovine manure fertilizer (Beuchat 1999), contaminated irrigation water (Wachtel *et al.* 2002), contaminated wash water or cross contamination during the cutting of the lettuce (Wachtel and Charkowski 2002).

Even though these contamination levels are low, foodborne outbreaks linked to the consumption of lettuce do occur (Ackers *et al.* 1998; Hilborn *et al.* 1999). As cut lettuce can harbor and support the growth of foodborne pathogens (nutrients from plant cellular material leakage), the efficacy of various decontamination methods was studied and reported. Among these reports, researchers identified two other pathogenic groups of microorganisms (*Listeria* spp. and *Pseudomonas* spp.) that are known to grow at the storage temperature (4C) used for cut lettuce products, making refrigeration as the sole means of controlling microbial growth tenuous (Beuchat and Brackett 1990). Many of the methods studied combined refrigeration (4C) with modified atmosphere packaging (MAP), water washing, chemical sanitizer wash or gaseous treatment, but with little success in eliminating the pathogen (Barriga *et al.* 1991; Soriano *et al.* 2000; Rodgers *et al.* 2004; Sy *et al.* 2005). In their report, Adams *et al.* (1989) showed that the uneven surface of the lettuce leaf could prevent the water wash or chemical sanitizer from reaching the pathogen. Takeuchi and Frank (2000, 2001) observed the bacteria cells within the stomata, the lettuce tissues and cuticle. These authors concluded that if a pathogen cell is located in the uneven lettuce surface folds or within the plant's tissue, these structures would protect any bacterial cell from sanitizer inactivation or wash removal.

The nonthermal process of ionizing irradiation is capable of penetrating the entire food product that is being treated. Using ionizing irradiation at low doses (<4 kGy) on fresh produce, researchers have reported bacterial reduction and improved shelf life while maintaining the sensory quality of the product (King *et al.* 1991; Farkas *et al.* 1997; Hagenmaier and Baker 1997; Prakash *et al.* 2000b; Rajkowski and Thayer 2000; Fan *et al.* 2003; Rajkowski *et al.* 2003). Using combinations of water rinse and irradiation treatments, we determined the microbiologic quality of washed fresh-cut lettuce samples that were rinsed in either warm (47C) or cold (5C) water before being irradiated at 1 or 2 kGy and stored up to 12 days at refrigeration temperature (4C).

MATERIAL AND METHODS

Lettuce Preparation

Individually wrapped iceberg lettuce (California cv. Sharpshooter) was purchased from a local distributor and refrigerated (3 ± 1C) overnight. The boxed lettuce was brought into a cold processing room (4 ± 1C) that had been previously cleaned and sanitized (Cleaning and Sanitizing Procedure, Proctor and Gamble, Cincinnati, OH). After the plastic wrap, outer leaves and cores were removed and discarded, the leaves were cut by hand into approximately 3-cm^2 pieces, mixed and randomly divided into two portions for rinsing at the different temperatures. Each half contained approximately 4 kg of cut lettuce and was washed in 100 L of distilled water in a water bath with a built-in circulator. The two water baths (warm and cold) were cleaned and sanitized between uses. One half, the warm water washed sample, was processed by placing the cut lettuce in a mesh basket and submerging for 2 min in warm water (48C), maintained within 0.5C using a heater (model MW-1140A-1, Blue M, Blue Island, IL), followed by a 1-min dip in the cold water (5C) rinse. The second half of the cut lettuce was placed in a mesh basket and submerged in cold water (5C) for 3 min. The total water wash contact time was 3 min for both warm and cold treatments. The washed lettuce was drained and spun dry using a hand-operated spinner (Wilton Industries Inc., Woodridge, IL) and mixed to provide a homogeneous sampling before sample collection and bagging.

Aseptic technique was used to obtain all control samples for microbial analysis. Untreated cut, cold water-dipped and warm water-dipped/cold rinse lettuce samples were taken. Water from the cold water, the warm water and the cold rinse water tanks were also obtained.

Lettuce samples weighing 150 g were placed in multilayer ethylene vinyl acetate/polyethylene bags with an O_2 transmission rate of 4,000 $cm^3/h/m^2$ at 23C (E-300, Cryovac, Deerfield, IL). The unsealed bagged samples were refrigerated (3 $\pm$ 1C) overnight before being heat sealed using an impulse sealer. Complete sample preparation details were presented earlier (Fan *et al.* 2003).

Irradiation of Lettuce

The bags containing the stored (overnight at 3 $\pm$ 1C) lettuce samples were heat sealed then irradiated at 0, 1 and 2 kGy at 4 $\pm$ 2C. A ^{137}Cs self-contained gamma radiation source (Lockheed Georgia Co., Marietta, GA) with a dose rate of 0.098 kGy/min was used. The dose rate was established using National Institute of Standards Technology (Gaithersburg, MD) alanine transfer dosimeters and verified using an electron paramagnetic resonance analyzer (EMS 104 EPR, Brucker, Rheinstellen, Germany). The sample temperature was monitored and maintained at 4 $\pm$ 2C by injecting the gas phase from liquid nitrogen into the irradiation chamber. After irradiation, the samples were stored and refrigerated (3 $\pm$ 2C) until use. The study was repeated twice.

Microbiologic Sample Preparation

The wash water and control lettuce samples were processed the same day they were collected. Three 25-g samples of the before and after washed lettuce were placed into individual stomacher bags. After a 1:10 dilution in 1.0% buffered peptone water ([BPW] BD/Difco, Sparks, MD) was prepared, the lettuce samples were stomached for 1 min at normal speed (Stomacher 400, Tekmar, Cincinnati, OH). BPW was used for the serial dilutions of both the lettuce and wash water samples.

Before the sealed bagged lettuce samples were irradiated, three random bags were opened and the control samples were taken. Thereafter, on the day of the microbial analysis, four sample bags of each irradiation treatment were chosen randomly from the stored samples. Three sample packages from each treatment were opened aseptically and 25-g samples were removed and placed into individual stomacher bags. The samples were kept on ice until they were brought to the microbiologic laboratory for analysis. The remaining samples were used for the sensory and quality determinations (Fan *et al.* 2003).

Microbiologic Analysis

Total aerobic, *Escherichia coli*/coliform and Enterobacteriaceae counts were performed using Petrifilm aerobic, coliform/*E. coli* and Enterobacteriaceae count plates (3M Microbiology, St. Paul, MN) following the AOAC directions for incubation time (24 h) and temperature (35 ± 2C). Duplicate plates were done for each sample. The Petrifilms were hand counted.

Statistical Analysis

Analysis of variance for significant differences at $P < 0.05$ (Excel, Microsoft Corp., Richmond, WA) was performed by comparing the data obtained from the water samples, the warm water- and cold water-washed lettuce and irradiated and nonirradiated treated lettuce samples.

RESULTS AND DISCUSSION

The microbial counts were recovered from the cold water and warm followed by cold rinse waters from two independent trials and are presented in Table 1. The aerobic counts from the cold water wash of trial 2 were statistically ($P < 0.05$) higher than the counts recovered from the cold water for trial 1, although the aerobic counts for the unwashed lettuce used in trial 2 was statistically lower ($P < 0.05$) than the cut lettuce used in trial 1 (Table 2). There was no difference in the coliform and Enterobacteriaceae counts recovered from the cold wash water. When comparing the warm water followed by the rinse washes for trials 1 and 2, there was no significant difference in the aerobic counts. However, trial 1 coliform and Enterobacteriaceae recovery counts recovered from the warm and rinse wash waters were statistically higher ($P < 0.05$) than the trial 2 coliform and Enterobacteriaceae population.

The differences between the trials in the recovered microbial counts from the wash water could be because of the condition of the two lettuce lots used. King *et al.* (1991) and Adams *et al.* (1989) reported that the outer leaves of the lettuce had higher microbial counts than the inner leaves. As the lettuce used in this study was purchased and processed at different times, the number of outer leaves removed before cutting and washing would affect the counts recovered in the wash water. The condition of the inner leaves (both dirt and

TABLE 1.
POPULATION OF TOTAL AEROBIC, COLIFORM AND ENTEROBACTERIACEAE RECOVERED FROM THE LETTUCE WASH WATER

Trial		Population (log cfu/mL)		
		Aerobic	Coliform	Enterobacteriaceae
1	Cold water†	2.6^{A}	1.9^{D}	1.2^{F}
	Warm water‡	4.7	3.6^{BD}	3.0^{CF}
	Rinse§	>2.0*	2.9	2.8
2	Cold water†	4.1^{A}	1.4^{E}	1.3^{G}
	Warm water‡	4.6	2.0^{BE}	2.3^{CG}
	Rinse§	2.8	2.0	0.7

$^{A–G}$ Values with same letter are statistically different at $P < 0.05$.
* Results are an estimate based on the TNTC (too numerous to count) procedure.
† Cold water at 5C after washing the lettuce for 3 min.
‡ Warm water at 47C after washing the lettuce for 2 min.
§ Cold water rinse at 5C for warm water-washed lettuce after rinsing for 1 min.

TABLE 2.
POPULATION OF TOTAL AEROBIC, COLIFORMS AND ENTEROBACTERIACEAE RECOVERED FROM CUT LETTUCE

Trial	Sample	Population (log cfu/g)		
		Aerobic	Coliforms	Enterobacteriaceae
1	Unwashed cut lettuce†	5.66 ± 0.04^{A}	5.01 ± 0.08^{A}	2.97 ± 0.10^{AC}
	Cold water washed‡	4.92 ± 0.07	4.3 ± 0.03	3.8 ± 0.09^{D}
	Bagged – 24 h	4.92 ± 0.22	4.38 ± 0.18	3.85 ± 1.5
	Warm water washed§	5.02 ± 0.20	4.3 ± 0.30	3.5 ± 0.30^{D}
	Bagged – 24 h	4.61 ± 0.61	2.62 ± 0.39	3.35 ± 0.10
2	Unwashed cut lettuce†	4.0 ± 0.01^{B}	4.0 ± 0.01^{B}	2.0 ± 0.01^{BE}
	Cold water washed‡	3.63 ± 0.70	3.86 ± 0.7	3.5 ± 0.70^{F}
	Bagged – 24 h	3.94 ± 0.21	3.68 ± 0.15	3.57 ± 0.18
	Warm water washed§	4.0 ± 0.01	>3.0*	4.0 ± 0.01^{CF}
	Bagged – 24 h	3.97 ± 0.21	3.42 ± 0.15	3.65 ± 0.20

$^{A–F}$ Values with the same letter are significantly different at $P< 0.05$.
* Results are an estimate based on the TNTC (too numerous to count) procedure.
† Untreated cut lettuce.
‡ Cold water at 5C washed for 3 min before bagging.
§ Warm water at 47C washed for 2 min then rinsed in cold water at 5C for 1 min.

microbial) was not exactly the same, and these differences were reflected by the different levels of recovered microorganisms.

A comparison was made of the recovered microbial population from the cold wash water (3-min contact time) versus the warm wash water (2-min contact time) within each trial (Table 1). The total coliform and Enterobacteriaceae from the warm water were higher ($P < 0.05$) even though the dipping time was shorter.

Presented in Table 2 are the results of the microbial population on the untreated, warm and cold water washed, and bagged and refrigerated (4C) for 24 h for the two independent trials. Comparing the total aerobic counts for the untreated cut lettuce samples, we observed a 1.6 and 1 log cfu/g difference for the total aerobic and coliform counts, respectively. The recovered count from trial 2 was lower, and this observed difference in the microflora was statistically significant ($P < 0.05$). Even though there was a difference in the microflora between the two batches of lettuce, the recovery of total aerobic, coliform and Enterobactericeae counts on the fresh-cut lettuce is within the reported range (Langerak 1978; Soriano *et al.* 2000). Soriano *et al.* (2000) reported a range of 3.0–7.8 log and <0.47 to >3.38 log for mesophilic aerobic and total coliform counts, respectively, on cut lettuce. The background counts on fresh-cut lettuce can vary because of the differences in growing conditions, harvesting, processing and packaging (King *et al.* 1991; Beuchat 1996). As the initial counts between the two trials varied, our results were not averaged but were compared.

After the cut lettuce was washed in the 5 or 48C water for both trials (Table 2), there was <l-log reduction in the aerobic or coliform counts, and this reduction was not statistically significant ($P < 0.05$). Li *et al.* (2001) washed cut lettuce with warm (50C) water for 90 s and reported a reduction in the initial mesophilic aerobic population of about 1.5 log cfu/g. In another study, Li *et al.* (2002) concluded that while the warm water treatment did reduce the initial bacterial population and delay browning, the warm water treatment could facilitate the growth of pathogens, such as *Listeria monocytogenes*, during refrigerated storage. Nascimento *et al.* (2003) reported >0.14-log reduction in the initial total coliform population after washing the lettuce leaves in running tap water for 1 min, which compares with our findings.

In both trials, we observed an increase in the Enterobacteriaceae counts on the lettuce after being washed in either warm or cold water (Table 2), but only the Enterobacteriaceae counts from trial 2 counts were statistically higher than the unwashed lettuce samples ($P < 0.05$). This increase in Enterobacteriaceae counts could be because of cross contamination from the wash water, which acts like an inoculum. Wachtel and Charkowski (2002) reported a 100% contamination of cut lettuce stored in inoculated water stored for 24 h at either room temperature or 4C. We observed that after being stored at 4C overnight, there was no change in the recovered total aerobic, total coliforms and Enterobacteriaceae counts.

As the counts of the stored bagged samples for the two trials were different and the amount of reduction after irradiation was similar, the results

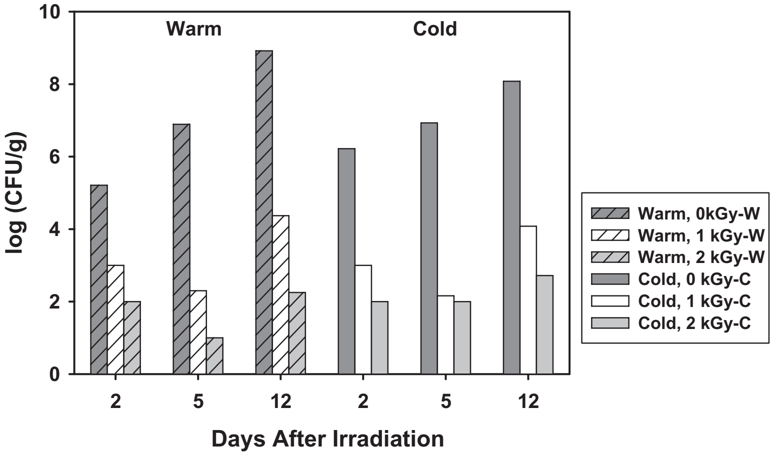

FIG. 1. TOTAL PLATE COUNTS OF WATER-WASHED CUT LETTUCE AT 5 AND 48C BEFORE AND AFTER IRRADIATION AT 1 AND 2 kGy

were not averaged, and we will discuss the results for trial 1 in this report. The total aerobic and coliform counts for the warm- and cold-treated bagged cut lettuce were reduced by 2 and 3 log for the 1- and 2-kGy treatments, respectively (Figs. 1 and 2). Upon storage at 4C for 12 days, the aerobic counts for the irradiated warm and cold washed and the coliform counts for the cold-washed lettuce increased but not to the initial level of the control (0 day sample), whereas the coliform counts for the irradiated (l and 2 kGy) warm water-washed samples increased to the level or exceeded the level of the control. After irradiation (1 and 2 kGy), the total Enterobacteriaceae counts were reduced by 2 for both the cold- and warm-washed samples (Fig. 3). These results compare well with the bacterial reduction observed after the low-dose irradiation of alfalfa sprouts (Rajkowski and Thayer 2001), broccoli sprouts (Rajkowski *et al.* 2003), cut peppers and carrots (Farkas *et al.* 1997), endive (Langerak 1978) and cut lettuce (Li *et al.* 2001).

Using the same prepared samples for the keeping quality study, we observed that the microbial reduction was better maintained when the samples were irradiated at 2 kGy and stored at 4C even though the reported keeping quality was better for the samples irradiated at 1 kGy (Fan *et al.* 2003). In their study, Rajkowski and Thayer (2000) reported a radiation destruct value range from 0.4 to 0.54 kGy for *Salmonella* and 0.30 to 0.34 kGy for *E. coli* O157:H7 on alfalfa, broccoli and radish sprouts and reported no differences in the D-10 radiation values among the various sprouts used. However, they did find that the destruct values differed significantly ($P < 0.05$) for the *Salmonella* isolate studied. Niemira (2003) observed a range in radiation destruct values

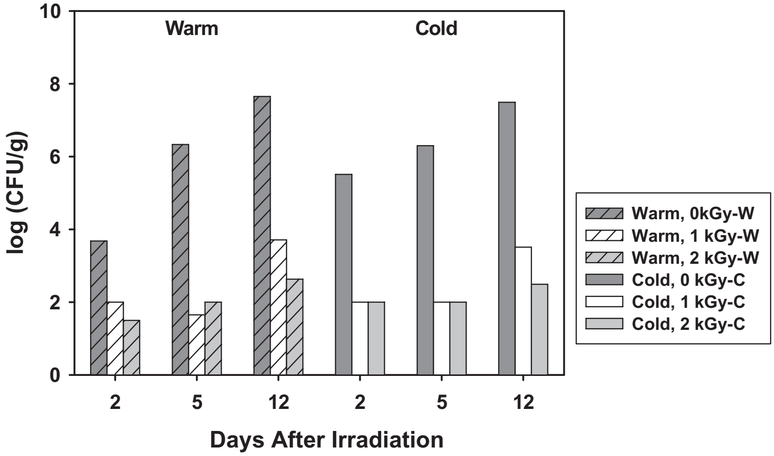

FIG. 2. TOTAL COLIFORM COUNTS OF WATER-WASHED CUT LETTUCE AT 5 AND 48C BEFORE AND AFTER IRRADIATION AT 1 AND 2 kGy

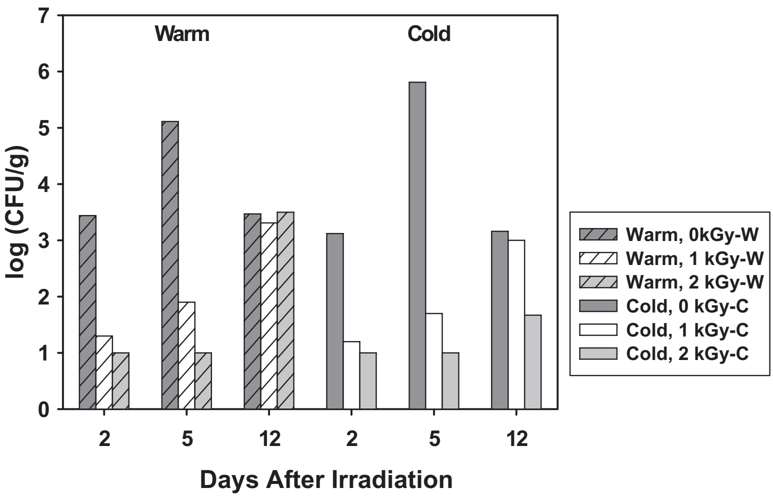

FIG. 3. TOTAL ENTEROBACTERIACEAE COUNTS OF WATER-WASHED CUT LETTUCE AT 5 AND 48C BEFORE AND AFTER IRRADIATION AT 1 AND 2 kGy

(0.24–0.31 kGy) for *Salmonella*, but did report a significant difference ($P < 0.05$) between the varieties of leaf lettuce used. Based on the reported D-10 irradiation values ranging from 0.24 to 0.54 kGy for *Salmonella* on fresh produce, a 5-log reduction of the produce-related isolate would not be achieved at the 1-kGy dose, and a higher dose would be necessary. In this present study, the 2-kGy dose achieved lower bacterial counts and about a 4-log reduction of *Salmonella* on the fresh-cut lettuce for both trials with a slightly lower keeping quality than the 1-kGy dose, which would only achieve about a 2-log reduction of *Salmonella*. Prakash *et al.* (2000b) reported that a 1-kGy dose eliminated the inoculated *E. coli* while increasing the shelf life of cut celery. Using a combined chlorination and 0.55-kGy irradiation treatments, Foley *et al.* (2002) reported that this combination achieved the >5-log reduction for *E. coli* O157:H7 and improved shelf life without adversely affecting the sensory properties of the cut lettuce.

The washed, cut lettuce was sealed in modified atmosphere film bags, where there was an increase in CO_2 levels and a decrease in O_2 levels because of the respiration of the lettuce (Fan *et al.* 2003). During the storage at 4C in the modified atmosphere sealed bags, the washed unirradiated lettuce maintained their sensory keeping quality (texture and nutrient content) (Fan *et al.* 2003). However, the modified atmosphere did not reduce the microbial counts as observed by a steady increase in aerobic and coliform counts for the unirradiated samples during storage (Figs. 1 and 2). These results of increased shelf life with no difference (maintenance or reduction) in microbial counts compare with those observed and reported by Barriga *et al.* (1991) and Prakash *et al.* (2000a), where similar CO_2/O_2 levels of modified atmosphere were used. When chlorination, MAP and low-dose irradiation of fresh-cut lettuce were combined, Hagenmaier and Baker (1997) reported that the microbial population was reduced and maintained for up to 8 days after irradiation. It was only after the irradiation treatment used in this study that the microbial counts were reduced.

In conclusion, the lower irradiation dose maintained the quality and storage properties of the packaged washed cut lettuce, but better microbiologic control was achieved at the 2-kGy level, which would give added pathogen reduction. The research data indicate that the variety of cut lettuce used can influence the radiation sensitivity of the pathogen and that the individual isolates influence the range of D-10 irradiation values. Studies should continue to determine the efficacy of introducing a sanitizer in the cold wash water coupled with low-dose irradiation and MAP in order to provide a pathogen-free product while maintaining the quality and storage properties of fresh-cut produce.

ACKNOWLEDGMENTS

We thank Kimberly J.B. Sokorai, Gina Bates, Amy Grant and Dana Drazul for their technical assistance.

REFERENCES

ACKERS, M.-L., MAHON, B.E., LEAHY, E., GOODE, B., DAMROW, T., HAYES, P.S., BIBB, W.F., RICE, D.H., BARRETT, T.J., HUTWAGNER, L. *et al*. 1998. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. J. Infect. Dis. *177*, 1588–1593.

ADAMS, M.R., HARTLEY, A.D. and COX, L.J. 1989. Factors affecting the efficacy of washing procedures used in the production of prepared salads. Food Microbiol. *6*, 69–77.

BARRIGA, M.I., TRACHY, G., WILLEMOT, C. and SIMARD, R.E. 1991. Microbial changes in shredded iceberg lettuce stored under controlled atmosphere. J. Food Sci. *56*, 1586–1588, 1599.

BEUCHAT, L.R. 1996. Pathogenic microorganisms associated with fresh produce. J. Food Prot. *59*, 204–216.

BEUCHAT, L.R. 1999. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces applied to lettuce and the effectiveness of chlorinated water as a disinfectant. J. Food Prot. *62*, 845–849.

BEUCHAT, L.R. and BRACKETT, R.E. 1990. Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. J. Food Sci. *55*, 755–758, 870.

FAN, X., TOIVONEN, P.M.A., RAJKOWSKI, K.T. and SOKORAI, K.J.B. 2003. Warm water treatment in combination with modified atmosphere packaging reduces undesirable effects of irradiation on the quality of fresh-cut iceberg lettuce. J. Agric. Food Chem. *51*, 1231–1236.

FARKAS, J., SÁRAY, T., MOHÁCSI-Farkas, C., HORTI, K. and ANDRÁSSY, E. 1997. Effects of low-dose gamma radiation on shelf-life and microbiological safety of pre-cut/prepared vegetables. Adv. Food Sci. *19*, 111–119.

FOLEY, D.M., DUFOUR, A., RODRIGUEZ, L., CAPORASO, F. and PRAKASH, A. 2002. Reduction of *Escherichia coli* O157:H7 in shredded iceberg lettuce by chlorination and gamma irradiation. Radiat. Phys. Chem. *63*, 391–396.

HAGENMAIER, R.D. and BAKER, R.A. 1997. Low-dose irradiation of cut iceberg lettuce in modified atmosphere packaging. J. Agric. Food Chem. *45*, 2864–2868.

HILBORN, E.D., MERMIN, J.H., MSHAR, P.A., HADLER, J.L., VOETSCH, A., WOJTKUNSKI, C., SWARTZ, M., MSHAR, R., LAMBERT-FAIR, M., FARRAR, J.A. *et al*. 1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. Arch. Intern. Med. *159*, 1758–1764.

KING, A.D., JR., MAGNUSON, J.A., TÖRÖK, T. and GOODMAN, N. 1991. Microbial flora and storage quality of partially processed lettuce. J. Food Sci. *56*, 459–461.

LANGERAK, D.L. 1978. The influence of irradiation and packaging on the quality of prepacked vegetables. Ann. Nutr. Aliment. *32*, 569–586.

LI, Y., BRACKETT, R.E., SHEWFELT, R.L. and BEUCHAT, L.R. 2001. Changes in appearance and natural microflora on iceberg lettuce treated in warm, chlorinated water and then stored at refrigeration temperature. Food Microbiol. *18*, 299–308.

LI, Y., BRACKETT, R.E., CHEN, J. and BEUCHAT, L.R. 2002. Mild heat treatment of lettuce enhances growth of *Listeria monocytogenes* during subsequent storage at 5C or 15C. J. Appl. Microbiol. *92*, 269–275.

NASCIMENTO, M.S., SILVA, N., CATAZONI, M.P.L.M. and SILVA, K.C. 2003. Effects of different disinfection treatments on the natural microbiota of lettuce. J. Food Prot. *66*(9), 1697–1700.

NIEMIRA, B.A. 2003. Radiation sensitivity and recoverability of *Listeria monocytogenes* and *Salmonella* on 4 lettuce types. J. Food Sci. *68*, 2784–2787.

PRAKASH, A., GUNER, A.R., CAPORASO, F. and FOLEY, D.M. 2000a. Effects of low-dose gamma irradiation on the shelf life and quality characteristics of cut romaine lettuce packaged under modified atmosphere. J. Food Sci. *65*, 549–553.

PRAKASH, A., INTHAJAK, P., HUIBREGTSE, H., CAPORASO, F. and FOLEY, D.M. 2000b. Effects of low-dose gamma irradiation and conventional treatments on shelf life and quality characteristics of diced celery. J. Food Sci. *65*, 1070–1075.

RAJKOWSKI, K.T. and THAYER, D.W. 2000. Reduction of *Salmonella* spp. and strains of *Escherichia coli* O157:H7 by gamma radiation of inoculated sprouts. J. Food Prot. *63*, 871–875.

RAJKOWSKI, K.T. and THAYER, D.W. 2001. Alfalfa seed germination and yield ration and alfalfa sprout microbial keeping quality following irradiation of seeds and sprouts. J. Food Prot. *64*, 1988–1995.

RAJKOWSKI, K.T., BOYD, G. and THAYER, D.W. 2003. Irradiation D-values for *Escherichia coli* O157:H7 and *Salmonella* sp. on inoculated broccoli seeds and effect of irradiation on broccoli sprout keeping quality and seed viability. J. Food Prot. *66*, 760–766.

RODGERS, S.L., CASH, J.N., SIDDIQ, M. and RYSER, E.T. 2004. A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe. J. Food Prot. *67*, 721–731.

SORIANO, J., RICO, M.H., MOLTÓ, J.C. and MAÑES, J. 2000. Assessment of the microbiological quality and wash treatments of lettuce served in university restaurants. Int. J. Food Microbiol. *58*, 123–128.

SY, K.V., MURRAY, M.B., HARRISON, M.D. and BEUCHAT, L.R. 2005. Evaluation of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and yeasts and molds on fresh and fresh-cut produce. J. Food Prot. *68*, 1176–1187.

TAKEUCHI, K. and FRANK, J.F. 2000. Penetration of *Escherichia coli* O157:H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. J. Food Prot. *63*, 434–440.

TAKEUCHI, K. and FRANK, J.F. 2001. Quantitative determination of the role of lettuce leaf structures in protecting *Escherichia coli* O157:H7 from chlorine disinfection. J. Food Prot. *64*, 147–151.

UNITED STATES DEPARTMENT OF AGRICULTURE, ECONOMIC RESEARCH SERVICE. 2005. U.S. Lettuce Statistics. http://www.ers.USDA.gov/data/foodconsumption/spreadsheets/vegfr.XLS (accessed October 27, 2006).

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, FOOD AND DRUG ADMINISTRATION. 2003a. FDA survey of domestic fresh produce. http://www.cfsan.fda.gov/~dms/prodsur10.html (accessed September 13, 2001).

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, FOOD AND DRUG ADMINISTRATION. 2003b. FDA survey of imported fresh produce. http://www.cfsan.fda.gov/~dms/prodsur6.html (accessed September 13, 2001).

WACHTEL, M.R. and CHARKOWSKI, A.O. 2002. Cross-contamination of lettuce with *Escherichia coli* O157:H7. J. Food Prot. *65*, 465–470.

WACHTEL, M.R., WHITEHAND, L.C. and MANDRELL, R.E. 2002. Association of *Escherichia coli* O157:H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water. J. Food Prot. *65*, 18–25.